



RECEIVED

JUN 21 2001

TECH CENTER 1600/2890

B. Webb
6/25/01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Kazuhisa HATAKEYAMA

Serial No. 09/576,715

Filed May 23, 2000

METHOD FOR GENE ANALYSIS

:
: Docket No. 2000-0644A
: Group Art Unit 1655
: Examiner B. Forman

THE COMMISSIONER IS AUTHORIZED
TO CHARGE ANY DEFICIENCY IN THE
FEES FOR THIS PAPER TO DEPOSIT
ACCOUNT NO. 23-0975

SUPPLEMENTAL AMENDMENT

Assistant Commissioner for Patents,
Washington, D.C. 20231

Sir:

Please amend the above-identified application as follows.

In the Specification:

Page 16, replace the paragraph beginning at line 16 with the following paragraph:

C1
The hybridization can be performed in the same manner as the usual nucleic acid hybridization, except that the hybridization is performed in the presence of a double-stranded DNA-binding protein. Specifically the hybridization can be performed as follows. First reducing agents such as dithiothreitol (DTT) and 2-mercaptoethanol, bovine serum albumin (BSA) and skim milk which prevent protein from non-specific binding to vessel and stabilize protein are added, and further protein accessory factors such as magnesium chloride ($MgCl_2$), salt-condensation regulators such as sodium chloride (NaCl) and potassium chloride (KCl) and so forth are added as required into buffer such as Tris buffer, phosphate buffer, citric acid buffer,

C1
cont

TES buffer, HEPES buffer or the like. The double-stranded DNA binding protein is then added to the solution. In this hybridization solution, the aforementioned oligonucleotide-immobilized nylon membrane (DNA (macro) array) and a labeled sample DNA are hybridized preferably for 1-20 minutes within a range of 40-120°C. When Sso7d is used as the double-stranded DNA binding protein, to a Tris buffer, 0.1-100 mM of DTT, 0.1-100 mM of MgCl₂ and 1-100 µg/µl of BSA (all of the concentrations are final concentrations) are added, and the Sso7d protein is added to the solution within a range of 0.001-10%. In this hybridization solution, the oligonucleotide-immobilized nylon membrane (DNA (macro) array) and a labeled sample DNA are hybridized preferably for 1-15 minutes within a range of 40-70°C.

Page 17, replace the paragraph beginning at line 18 with the following paragraph:

C2

After the hybridization reaction, the membrane was washed with buffer such as Tris buffer, phosphate buffer, citric acid buffer, TES buffer, HEPES buffer or the like for 1-10 minutes within a range of 10-50°C, and then dried. In this case, adding a small amount of surfactant such as sodium dodecyl sulfate (SDS) is preferable because it can prevent from non-specific binding and step down background. When Sso7d is used as the double-stranded DNA binding protein, after the hybridization reaction, it is preferable that the membrane should be washed with citric acid buffer such as SSC, more preferably buffer added with SDS within a range of 0.001-0.05% as required to SSC, for 3-10 minutes within a range of 10-40°C, and then air-dried. As for the hybridization signal, radiation dose or the like of each dot on the dried nylon membrane can be measured by autoradiography etc. to calculate the hybridization strength.

Page 20, replace the paragraph beginning at line 3 with the following paragraph:

C³ Further, a test kit for using to perform the aforementioned gene analysis can be prepared by using a double-stranded DNA-binding protein. Such a test kit is constituted by components similar to those of ordinary test kits for gene analysis utilizing hybridization except for the use of the double-stranded DNA-binding protein. That is, the test kit of the present invention comprises at least a double-stranded DNA-binding protein and, as optional components, washing solution, diluent, hybridization solution and so forth.

Page 22, replace the paragraph beginning at line 15 with the following paragraph:

C⁴ A Biotyne C (trademark, produced by Pall) membrane was rinsed with 0.1 N HCl to acidify it, and immersed in 20% EDC (1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride) at room temperature for 15-30 minutes. The membrane was lightly rinsed with deionized water and 0.5 M sodium hydrogencarbonate buffer (pH 8.4), then mounted on a dot blot apparatus (produced by Bio-Rad) and allowed to react with the amino-modified oligonucleotide (1) or (2), which was suspended in a 0.5 M sodium hydrogencarbonate buffer (pH 8.4), at room temperature for 15 minutes.

Page 31, replace the paragraph beginning at line 21 with the following paragraph:

C⁵ The hybridization signal was evaluated by measuring amount of fluorescent dye of each spot on the air-dried slide glass by using ScanArray 3000 produced by General Scanning to calculate the hybridization signal strength. The results are shown in Table 5. The results are represented with relative values based on the hybridization signal strength of the probe DNA (4) obtained with the addition of Sso7d, which is taken as 100.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Applicants have effected additional amendments to the specification to correct some spelling errors in the specification. All the changes are minor in nature and are self-explanatory.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

Respectfully submitted,

Kazuhisa HATAKEYAMA

By: _____

Lee Cheng
Registration No. 40,949
Attorney for Applicant

LC/gtn
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
June 18, 2001